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Effect of hydrophobic chain length of the chiral surfactant on enantiomeric separations by electrokinetic chromatography: Comparison between micellar and vesicular pseudo-stationary phases

Ashok Mohanty, Joykrishna Dey *

Department of Chemistry, Indian Institute of Technology, Kharagpur 721302, India

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Abstract

Two novel amino acid based surfactants sodium *N*-(4-*n*-decyloxybenzoyl)-L-valinate (SDeBV) and sodium *N*-(4-*n*-octyloxybenzoyl)-L-valinate (SOBV) have been synthesized and used as chiral selectors for enantiomeric separations by micellar electrokinetic chromatography (MEKC). The aggregation behavior of the surfactants was studied in buffered aqueous solution using surface tension and fluorescence probe techniques. The microenvironment of the aggregates was studied using pyrene, and 1,6-diphenyl-1,3,5-hexatriene (DPH) as probe molecules. Results of these studies indicate that these two surfactants form micelles in buffered aqueous solution. Successful enentioseparation has been achieved for 1,1′-bi-2-naphthol (BOH), 1,1′-binaphthyl-2,2′-diylhydrogenphosphate (BNP), 2,8-dimethyl-6H-5,11-methanodibenzo[*b*,*f*] [1,5]diazocine (Tröger's base, TB), and benzoin (BZN) using the two chiral selectors SDeBV and SOBV. The separations were optimized with respect to surfactant concentration, pH, and buffer concentration. The results are discussed in light of the aggregation behavior of the surfactants. A comparison of the results of this study has been made with the data from literature to investigate the effect of self-assembly morphology on enantiomeric separations.

Keywords: Capillary electrophoresis; MEKC; Enantiomeric separation; Hydrophobic chain length

1. Introduction

Enantiomeric separation of chiral molecules is important to environment and biological fields as well as to synthetic chemists and pharmaceutical industry. This is primarily because of the need for pure enantiomers in biomedical studies, asymmetric synthesis, catalysis, and medicines. Micellar electrokinetic chromatography (MEKC), the mode of capillary electrophoresis (CE) introduced by Terabe et al. [1] for analysis of neutral analytes, is an extensively used analytical technique for enantiomeric separations in recent years [2,3]. The technique involves use of a surfactant at a concentration above its critical micellar concentration (cmc) in the background electrolyte (BGE). The surfactant molecules self-assemble to form micelles that act as the pseudo-stationary phase. Chiral separation in MEKC is achieved by use of a chiral selector in the BGE in combination with an achiral surfactant like sodium dodecyl sulfate (SDS). Different chiral selectors used in MEKC include but not limited to cyclodextrins, macrocyclic antibiotics, crown ethers, and calixarenes, etc. [4–7]. An alternative way is to use a chiral surfactant above its cmc in the background electrolyte. The chiral surfactant form micelles with stereogenic centers at the surface and it acts as the chiral selector. A variety of chiral surfactants have been used in MEKC to achieve enantiomeric separation of a wide spectrum of compounds like chiral drugs, pesticides, atropisomeric compounds, and derivatized amino acids [8–25]. The chiral surfactants utilized are naturally occurring surfactants digitonin [8], saponin [9], and bile salts [10], glucopyranoside based sulfate and phosphate surfactants [11], amino acid based surfactants [12–19] and polymeric surfactants [20–25]. Among all the chiral surfactants, amino acid based monomeric and polymeric surfactants are most widely used. This is because of the advantages associated with the amino acid based surfactants. The advantages are (i) surfactants with a variety of chiral

* Corresponding author. Tel.: +91 3222 283308; fax: +91 3222 255303.
E-mail address: joydey@chem.iitkgp.ernet.in (J. Dey).

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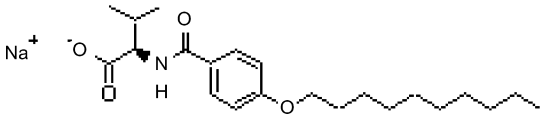
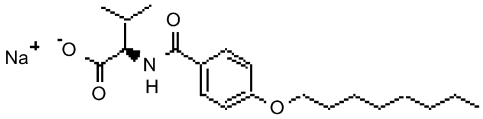
headgroups and chemical compositions can be synthesized to manipulate the chiral selectivities, and (ii) both D and L optical configurations of amino acid-based surfactants are available to determine enantiomeric impurities more accurately by reversal of migration order of the two enantiomers. In fact, a number of reports have appeared in the literature in which authors have used surfactants and polymers having different amino acids as headgroups to investigate the mechanism of chiral recognition [26–30]. The results of these studies indicate that chiral recognition occurs due to interaction of the analytes with the chiral selector near the stereogenic center, i.e. at the micellar surface. However, the effect of length of the hydrophobic tail of the surfactant on enantiomeric separations has not been reported so far. The change in length of the hydrophobic tail of the surfactant can lead to formation of different kind of aggregates by the surfactant molecules when dissolved in the BGE. This is because, hydrophobic interaction between the hydrocarbon tails of the surfactant molecules is one of the driving forces for aggregate formation and in most cases, it determines the extent of close packing of the surfactant molecules during aggregate formation. Therefore, difference in hydrophobic chain length will result in difference in shape, size, and physical properties like microviscosity, micropolarity of the self-assemblies formed. All these factors will have direct influence on the partitioning of the analyetes with the pseudo-stationary phase, which is the essential condition to obtain separation in electrokinetic chromatography (EKC).

In our recent works reported earlier [17–19], we have used the vesicle-forming amino acid based surfactants sodium *N*-(4-*n*-dodecyloxybenzoyl)-L-amino acidates having L-valine, L-leucine, and L-isoleucine (SDBV, SDBL, and SDBIL, respectively) as the headgroups for enantiomeric separations of 1,1′-bi-2-naphthol (BOH), 1,1′-binaphthyl-2,2′-diylhydrogenphosphate (BNP), 2,8-dimethyl-6H-5,11-methanodibenzo[*b*,*f*][1,5]diazocine (Tröger's base, TB), and benzoin (BZN) using EKC. The vesicular pseudo-stationary phase has been shown to provide very large migration window, good chiral selectivity, and resolution for the above compounds. We have also shown that the structure of the surfactant headgroup has significant effect on enantiomeric separations [19]. In this work, two new surfactants sodium *N*-(4-*n*-decyloxybenzoyl)-L-valinate (SDeBV), and sodium *N*-(4-*n*-octyloxybenzoyl)-L-valinate (SOBV) have been synthesized and used as chiral selectors for enantiomeric separation of BOH, BNP, BZN, and TB (see Chart 1 for molecular structures). The surfactants SDeBV and SOBV have ten and eight carbon atoms, respectively, in the hydrocarbon chain and both have L-valine as the hydrophilic headgroup. The purpose of this study is to evaluate the effect hydrophobic chain length of the surfactants on enantiomeric separations. Before using in EKC, the aggregation behavior of the surfactants was studied in borate buffer to know the type of aggregates formed by these surfactants. It was found that unlike SDBV, which form vesicles, both SDeBV and SOBV form micelles in borate buffer. Nevertheless, successful enantiomeric separations were obtained for the said analytes using both SDeBV and SOBV. The chromatographic results obtained for the micellar and vesicular systems have been compared.



Sodium N-(4-n- decyloxybenzoyl)- L-valinate (SDeBV)



Sodium N-(4-n-octyloxybenzoyl)- L-valinate (SOBV)

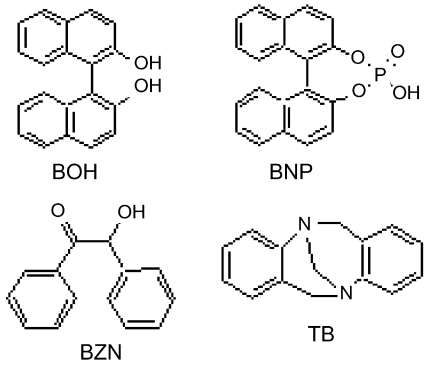


Chart 1. Molecular structures of the surfactants and analytes.

2. Experimental

2.1. Materials

The racemic mixtures and pure enantiomers of 1,1′-bi-2-naphthol, 1,1′-binaphthyl-2,2′-diylhydrogenphosphate, 2,8-dimethyl-6H-5,11-methanodibenzo[*b*,*f*] [1,5]diazocine (Tröger's base), and benzoin were purchased from Sigma (St. Louis, MO, USA) and Aldrich (Milwaukee, WI, USA). The fluorescence probes pyrene and 1,6-diphenyl-1,3,5-hexatriene (DPH) were obtained from Aldrich and recrystallized thrice from acetone–ethanol mixture before use. Dodecanophenone was obtained from Aldrich and sodium tetraborate was purchased from SRL (Mumbai, India). Fused silica capillary was obtained from Polymicro Technologies (Phoenix, AZ, USA). The surfactants SDeBV and SOBV were synthesized and purified in the laboratory using reported procedure [17,18,31].

2.2. Instrumentation

The Prince CE system (Prince Technologies, The Netherlands) equipped with an autosampler, Lambda 1010 variable wavelength UV–vis detector (Bischoff, Leonberg, Germany), and inbuilt temperature control system was utilized in this study. Uncoated fused silica capillary having 50 μm internal diameter and 87 cm total length (31.5 cm from inlet to detector) was used for all the separations. Data was collected and processed on a personal computer using Dax 7.0 data acquisition and analysis software. A digital pH meter model

pH5652 (Electronics corporation of India limited, Calcutta, India) with a glass electrode was used for pH measurements. Surface tension measurements were performed using a Du Nüoy ring tensiometer (S.D. Hudson & Co., Kolkata). A Perkin Elmer LS-55 luminescence spectrometer equipped with a filter polarizer and thermostated cell holder was used for fluorescence measurements. Temperature was controlled by use of a Neslab RTE 7 circulating bath (Thermo Neslab, USA).

2.3. Methods

Stock borate buffer solutions (100 ml) of desired concentration and pH were prepared by dissolving appropriate amount of solid sodium tetraborate decahydrate in Milli-Q water (18 MΩ resistivity) and adjusting the pH by addition of either dilute HCl or dilute NaOH. The surfactant solutions employed for separations were made by mixing weighed amount of solid surfactant in the appropriate buffer solution. The pH of the surfactant solutions in buffer was again measured and adjusted if required. The surfactant solutions were then filtered through a membrane filter of 0.45 μm pore size (Millipore, Bedfold, MA, USA) and degassed in a Bandelin Sonorex (Model RK 100 H) ultrasonic bath for 5 min prior to use. All sample solutions were prepared by dissolving 4 mg of solute in 2 ml methanol for stock solution and diluting this to a concentration of 0.4–0.6 mg/ml with buffer solution for analysis. The final sample contained 20–30% (v/v) methanol. For fluorescence measurements, stock solutions (1×10^{-3} M) of pyrene and DPH were made in methanol. Aliquots of this solution was added to 5 ml of the surfactant solution of desired concentration to make the final pyrene and DPH concentrations 1×10^{-6} and 5×10^{-6} M, respectively. Pyrene was excited at 335 nm and emission spectrum was recorded in the range of 350–500 nm. DPH was excited at 350 nm and the emission intensity was followed at 450 nm.

2.4. Electrophoretic procedure

The untreated fused silica capillary was first activated by purging with 1 M NaOH for 30 min followed by 0.1 M NaOH for additional 60 min. At the beginning of each day the capillary was first rinsed with 0.1 M NaOH for 30 min and with water for 30 min. For MEKC separations, the capillary was treated successively with 0.1 M NaOH, water, buffer and run buffer (surfactant solution in buffer) for 5 min each using 1000 mbar pressure before injection of a new sample. Between two successive runs of the same sample, the capillary was rinsed only with water and run buffer for 5 min each. The injection of the sample was done using a pressure of 20 mbar for 0.02 min. Separations were carried out by applying a constant voltage of 15–25 kV. Detection was performed at a wavelength of 230 nm. The surfactants has absorption maximum at 255 nm. At 230 nm the molar absorptivity of the surfactants is relatively low (4500 l mol^{-1} cm^{-1}) compared to that of the analytes (1,05,000 l mol^{-1} cm^{-1} for BOH). Due to the use of low concentration of surfactants, no difficulty was faced while detecting the analytes.

2.5. Calculations

Resolution (R_s) was calculated using the method involving peak width at half-height [18].

$$R_s = \frac{(2.35/2)(t_{r2} - t_{r1})}{W_{50(1)} + W_{50(2)}} \quad (1)$$

where t_{r1} and t_{r2} are the migration time and $W_{50(1)}$ and $W_{50(2)}$ are the peak width at 50% height of the 1st and 2nd isomer, respectively. The selectivity (α) was calculated from the ratio of the migration time of the two enantiomers. The t_{mc} values were measured using dodecanophenone as the micelle marker. The t_{mc} was measured to be 72.3 min for SDeBV and 43.6 min for SOBV in 50 mM borate buffer pH 9.7 with an applied voltage of 15 kV.

3. Results and discussion

3.1. Aggregation behavior of the surfactants in buffer solution

The aggregation behavior of the surfactants SDeBV and SOBV were investigated in buffered aqueous solution prior to their use in MEKC. The objective was to know the nature of the self-assemblies formed by these surfactants. The studies were performed in 50 mM borate buffer, pH 9.7. The critical aggregation concentration (cac) was determined by surface tension measurement method. Surface tension (γ) of a series of surfactant solutions made in borate buffer solution was measured and plotted against the surfactant concentration. The breakpoint in the plot of γ versus log (concentration) gave the cac value. The cac value thus obtained for SDeBV and SOBV are listed in Table 1. The cac value of SDBV in same buffer solution reported earlier [18] is also included in the table for comparison purpose. As expected, the cac value increases with the decrease of hydrophobic chain length. To investigate the microenvironment of the self-assemblies, fluorescence probe studies were performed using pyrene and DPH as extrinsic probe molecules. The ratio of intensities of first and third vibronic bands in the emission spectrum of pyrene (I_1/I_3) is known to be highly sensitive to the polarity of the region in which pyrene molecule is solubilized [32]. Due to highly hydrophobic nature, pyrene has very low solubility in water and gets solubilized inside the surfactant aggregates when dissolved in surfactant solutions above their cac. Thus, measurement of I_1/I_3 gives an indication about the micropolarity of the self-assemblies. The I_1/I_3 value of pyrene was measured in presence of 2 mM SDeBV and SOBV. The values are included in Table 1. The data presented in Table 1 reveal that the microenvironment of the self-assemblies

Table 1
Critical aggregation concentration (cac), polarity ratio (I_1/I_3), and fluorescence anisotropy (r) of DPH in SDeBV, SOBV, and SDBV surfactants

Surfactant	cac × 10^5 (M)	I_1/I_3	r
SDeBV	6.1	1.07	0.072
SOBV	50.2	1.09	0.064
SDBV	2.4	1.05	0.112

of SDeBV and SOBV is relatively more polar compared to that of SDBV. This may be due to the loose packing of the SDeBV and SOBV monomers in the self-assemblies, which allow the penetration of water molecules into the aggregate core resulting in an increase of micropolarity. The loose packing of the surfactant monomers in the self-assemblies of SDeBV and SOBV is further indicated by the low fluorescence anisotropy value (r) of DPH molecule measured in presence of these surfactants above their cac. DPH is a well-known microviscosity or more appropriately microfluidity probe and has been used for studying many surfactant self-assembly systems [33–35]. The r is an index of equivalent microviscosity in the surfactant aggregate core. The r-value measured in presence of 2 mM SDeBV and SOBV are tabulated in Table 1. Comparison of the r-values of the three surfactants indicates that the microenvironment of SDeBV and SOBV aggregates is less rigid compared to that of SDBV. In fact, the r-values obtained for SDeBV and SOBV are similar to the r-value measured for micelle forming surfactants sodium dodecyl sulfate (0.054) and sodium dodecylbenzene sulfonate (0.061). The low r-value and high I_1/I_3 ratio obtained for the self-assemblies of SDeBV and SOBV indicate that unlike SDBV, which form vesicles, these two surfactants form micelles in buffered aqueous solution. The formation of micellar structures by SDeBV and SOBV in buffer solution was further confirmed by the transmission electron microscopic study. No recognizable structures could be observed in the samples made from the solutions of SDeBV and SOBV. The shortening of the hydrophobic chain length in these surfactants results in weakening of the hydrophobic interactions among the surfactant tails during aggregate formation. This hinders the tight packing of the surfactant monomers and does not favor vesicle formation.

3.2. Enantiomeric separations

After characterizing the nature of self-assemblies formed by the surfactants SDeBV and SOBV in borate buffer solution, both the surfactants were used as chiral selectors for enantiomeric separation of the chiral compounds BOH, BNP, BZN, and TB. The method development procedure involves optimization of resolution (R_s) with respect to the analytical parameters like pH, buffer concentration, and surfactant concentration. The surfactants SDeBV and SOBV being sodium salts of carboxylic acids, get precipitated out of the solution at pH < 7.0. Therefore, all the separations were carried out in the alkaline pH range. Borate buffer was used as the background electrolyte as it has low conductivity and high buffer capacity in the studied alkaline pH range (8.5–10.3). First, the pH was optimized for enantiomeric separation of all the four analytes BOH, BNP, BZN, and TB using both SDeBV and SOBV as chiral selectors. A series of experiments were performed using 50 mM borate buffers having pH 8.5, 9.0, 9.3, 9.7, and 10.3 with either 2 mM SDeBV or 4 mM SOBV. The applied voltage was 15 kV for all the cases. From the pH optimization study (results not shown here) it was found that the optimum pH for enantiomeric separation of BOH is 9.7 and the optimum pH for all other three analytes BNP, BZN, and TB is 10.3. In order to find the optimum buffer concentration, separations were carried out individually for all the four analytes in the buffer concentration range of 30–70 mM using both SDeBV and SOBV. The pH of the borate buffer was 9.7 for BOH and 10.3 for BNP, BZN, and TB. The results of buffer concentration optimization study show that 50 mM borate buffer provides optimum resolution for BOH, BNP, and TB and 60 mM borate buffer gives the optimum separation for BZN using both SDeBV and SOBV. The optimized conditions of pH and buffer concentration obtained for enantiomeric separation of BOH, BNP, BZN, and TB are same as reported in the literature [18] for separation of these analytes using SDBV as chiral selector. This is expected because the molecular structures of the chiral selectors are same except the length of the hydrophobic tail. The difference in hydrophobic chain length, however, results in difference in cmc of the surfactants as shown by aggregation behavior study. Therefore, one expects the optimum surfactant concentration needed for separation of any particular analyte to be different for the three surfactants SDBV, SDeBV, and SOBV. The optimization of surfactant concentration for enantiomeric separation of each individual analyte is discussed below. The previously optimized conditions of pH and buffer concentration, i.e. 50 mM borate buffer pH 9.7 for BOH, 50 mM borate buffer pH 10.3 for BNP and TB, and 60 mM borate buffer pH 10.3 was used for this study.

3.2.1. Optimization of SDeBV concentration

The concentration of SDeBV was varied in the range 0.5–7.0 mM. The representative electropherograms showing the effect of SDeBV concentration on enantiomeric separation of BOH are shown in Fig. 1. The applied voltage was 25 kV. Similar

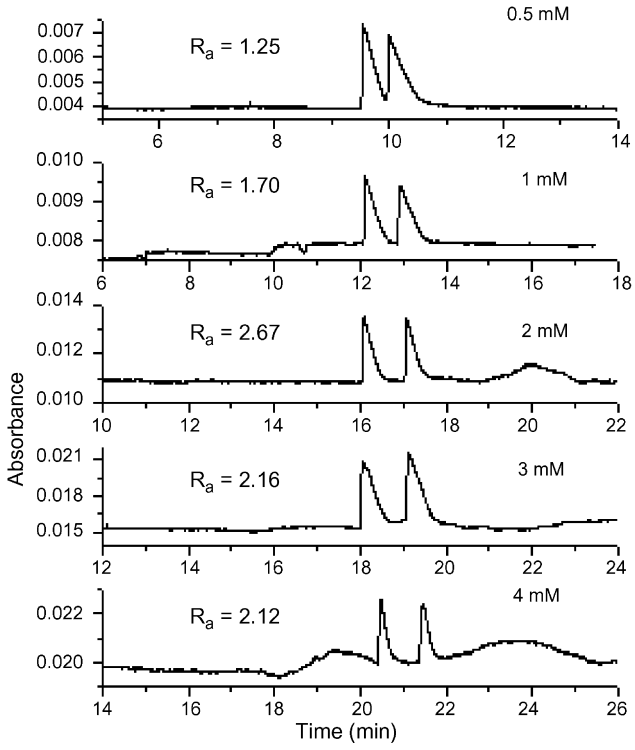


Fig. 1. Electropherograms showing effect of SDeBV concentration on enantiomeric separation of BOH. Separation condition: 50 mM borate buffer, pH 9.7 and the applied voltage is 25 kV.

Table 2
EOF migration time (t_o), migration time of first and second enantiomer (t_1 and t_2), selectivity (α), and resolution (R_s) values for enantiomeric separation of BOH, BNP, and BZN at different SDeBV concentrations

Analyte	[SDeBV] (mM)							
	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0
BOH[a]								
t_o	4.42	4.42	4.40	4.38	4.40	4.26	–	–
t_1	9.55	12.12	16.09	18.10	20.47	21.10	–	–
t_2	10.01	12.93	17.09	19.14	21.46	21.84	–	–
α	1.05	1.06	1.06	1.06	1.05	1.04	–	–
R_s	1.25	1.70	2.67	2.16	2.12	2.05	–	–
BNP[b]								
t_o	–	–	6.23	6.08	6.41	6.39	6.41	6.27
t_1	–	–	15.32	15.45	18.59	20.48	21.90	21.91
t_2	–	–	15.64	15.85	19.22	21.32	22.84	22.91
α	NS	NS	1.02	1.02	1.03	1.04	1.04	1.04
R_s	NS	NS	1.25	1.47	1.89	2.46	2.51	2.73
BZN[c]								
t_o	–	–	–	10.07	10.11	10.10	10.02	9.89
t_1	–	–	–	14.06	15.31	15.48	16.41	16.95
t_2	–	–	–	14.31	15.66	15.81	16.85	17.42
α	NS	NS	NS	1.01	1.02	1.02	1.02	1.03
R_s	NS	NS	NS	1.17	1.58	1.60	1.60	1.78

NS, no separation.
[a] Separation condition: 50 mM borate buffer pH 9.7, 25 kV.
[b] Separation condition: 50 mM borate buffer pH 10.3, 20 kV.
[c] Separation condition: 60 mM borate buffer pH 10.3, 15 kV.

concentration optimization studies were performed for all other analytes BNP, BZN, and TB. The applied voltage was 20 kV for the separations of BNP, and 15 kV for BZN and TB. The R_s values and other chromatographic parameters are included in Table 2. Values for TB are not included in the table because baseline separation could not be obtained using SDeBV. From Table 2 it can be observed that R_s value for BOH increases with the increase of surfactant concentration, reaches a maximum at 2 mM, and then starts falling again. Thus, the optimum SDeBV concentration for the separation of BOH is 2 mM. In case of BNP and BZN, R_s value increases with the increase of surfactant concentration up to 7 mM. SDeBV concentration higher than 7 mM could not be employed due to the detection problem caused by the significant absorbance of the surfactant. A minimum of 2 mM and 3 mM SDeBV is required for the enantiomeric separation of BNP and BZN, respectively. TB could not be baseline separated using SDeBV. The highest R_s value obtained is 1.26 using 4 mM SDeBV. The migration order of the two enantiomers was determined using spiking technique. The (*S*)-BOH migrates faster and hence interacts weakly with the SDeBV micelles compared to the (*R*) enantiomer. The migration order for BNP enantiomers was opposite. The (*R*)-BNP migrates faster compared to the (*S*)-BNP.

3.2.2. Optimization of SOBV concentration

The cmc of SOBV is 0.5 mM. For SOBV concentration optimization studies, concentration of SOBV was varied in the range of 1–7 mM. The representative electropherograms showing effect of SOBV concentration on enantiomeric separation

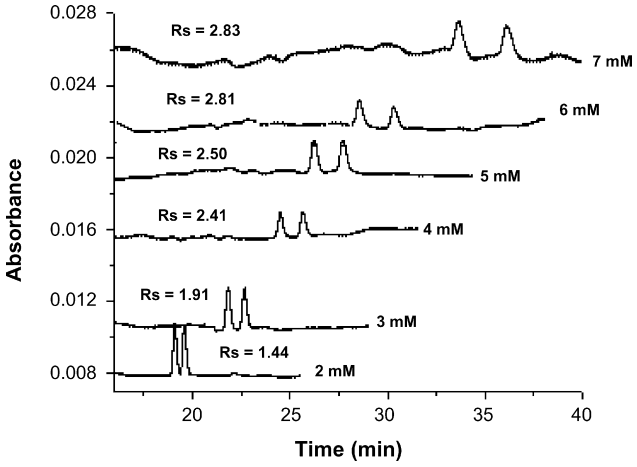


Fig. 2. Effect of SOBV concentration on enantiomeric separations of BNP. Separation condition: 50 mM borate buffer, pH 10.3. The applied voltage was 20 kV.

of BNP are presented in Fig. 2. The applied voltage was 20 kV. Similar studies were performed for all other analytes BNP, BZN, and TB. The applied voltage was 20 kV for BNP, and 15 kV for BZN and TB. The R_s values and other chromatographic parameters thus obtained in each case are included in Table 3. Due to lack of baseline separation, values for TB are not included in the table. The optimum SOBV concentration for separation of BOH is 3 mM, which is slightly higher than the optimum SDBV and SDeBV concentration required for separation of the said analyte. As expected, no separation could be achieved for BOH using 0.5 mM SOBV and only partial separation could

Table 3
EOF migration time (t_o), migration time of first and second enantiomer (t_1 and t_2), selectivity (α), and resolution (R_s) values for enantiomeric separation of BOH, BNP, and BZN at different SOBV concentrations

Analyte	[SOBV] (mM)						
	1.0	2.0	3.0	4.0	5.0	6.0	7.0
BOH[a]							
t_o	5.97	6.11	5.98	5.95	–	–	–
t_1	10.45	18.72	22.37	29.02	–	–	–
t_2	10.80	20.39	24.53	31.78	–	–	–
α	1.03	1.08	1.09	1.09	–	–	–
R_s	1.17	3.70	3.43	3.30	–	–	–
BNP[b]							
t_o	–	6.88	6.84	6.81	6.85	6.81	6.93
t_1	–	19.09	21.83	24.51	26.22	28.47	33.63
t_2	–	19.58	22.66	25.68	27.71	30.24	36.11
α	NS	1.02	1.04	1.05	1.05	1.06	1.07
R_s	NS	1.44	1.91	2.41	2.50	2.81	2.83
BZN[c]							
t_o	–	–	–	10.23	10.19	10.38	10.12
t_1	–	–	–	14.28	15.34	16.41	17.07
t_2	–	–	–	14.52	15.68	16.82	17.58
α	NS	NS	NS	1.02	1.02	1.02	1.03
R_s	NS	NS	NS	0.97	1.28	1.46	1.81

NS, no separation.
[a] Separation condition: 50 mM borate buffer pH 9.7, 20 kV.
[b] Separation condition: 50 mM borate buffer pH 10.3, 20 kV.
[c] Separation condition: 60 mM borate buffer pH 10.3, 15 kV.

be achieved using 1 mM SOBV owing to its higher cmc value. Resolution improved drastically when SOBV concentration was raised from 1 to 3 mM. Upon further increase of surfactant concentration R_s value decreases slightly due to broadening of the peaks. The minimum SOBV concentration required for separation of BNP and BZN are 2 and 4 mM, respectively. The R_s values increase continuously for both of these analytes with the increase of SOBV concentration up to 7 mM after which separations could not be carried out due to detection problem as discussed earlier. Therefore, 7 mM SOBV was considered to be the optimum surfactant concentration for these two analytes. The migration order of the two enantiomers was determined by spiking technique. As expected, the migration order does not change with the change in length of hydrophobic tail of the surfactants.

3.2.3. *Effect of hydrophobic chain length of surfactants on chiral separation*

In order to evaluate the effect of change of hydrophobic chain length on enantiomeric separations, the results obtained from the separations of BOH, BNP, BZN, and TB using SDBV, SDeBV, and SOBV were compared. It is to be noted that the change in the length of hydrophobic tail of the surfactants results in formation of different types of aggregates by these surfactants. SDBV forms vesicles [17,18] whereas both SDeBV and SOBV form micelles. Therefore, comparison of the results will help to gain knowledge about the effect of aggregate morphology on chiral selectivity of the chiral surfactants. For comparison, results obtained for any particular analyte using the same separation conditions (pH, buffer concentration, voltage, and optimum surfactant concentration) are considered. The conditions considered are 50 mM borate buffer, pH 9.7 containing 2 mM surfactant for BOH, 50 mM borate buffer, pH 10.3 containing 6 mM surfactant for BNP, 60 mM borate buffer, pH 10.3 with 6 mM surfactant for BZN, and 50 mM borate buffer, pH 10.3 with 4 mM surfactant for TB. The applied voltage was 15 kV in all the cases. The electropherograms for BOH, BNP, BZN, and TB using SDBV, SDeBV, and SOBV as chiral selectors are shown in Figs. 3 and 4. The chromatographic parameters

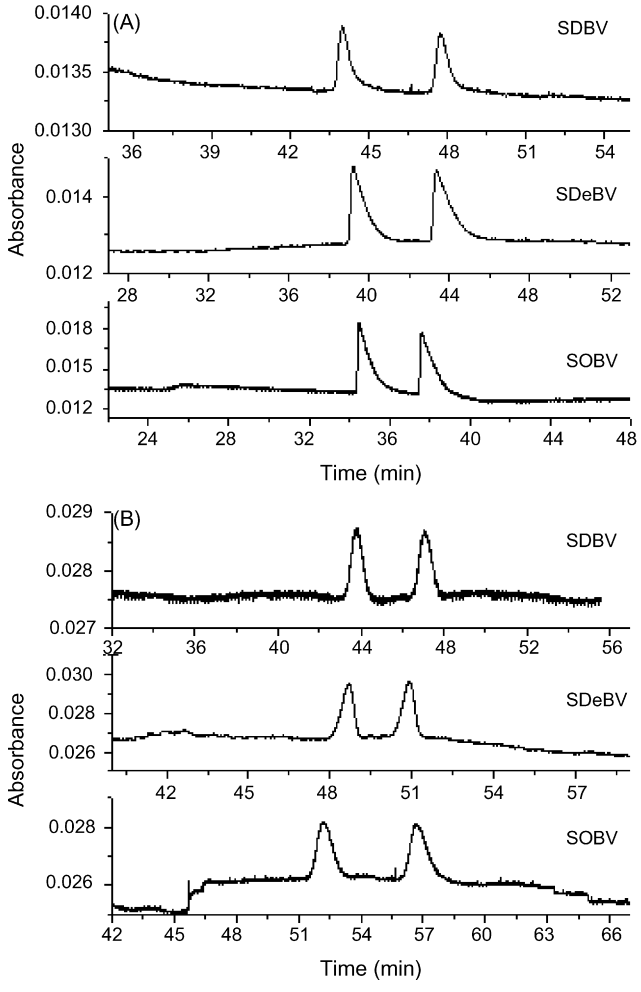


Fig. 3. Electropherograms obtained for enantiomeric separation of BOH (A) and BNP (B) using SDBV, SDeBV, and SOBV as chiral selectors. Separation conditions: 50 mM borate buffer, pH 9.7 with 2 mM surfactant for BOH and 50 mM borate buffer, pH 10.3 with 7 mM surfactant for BNP. The applied voltage is 15 kV.

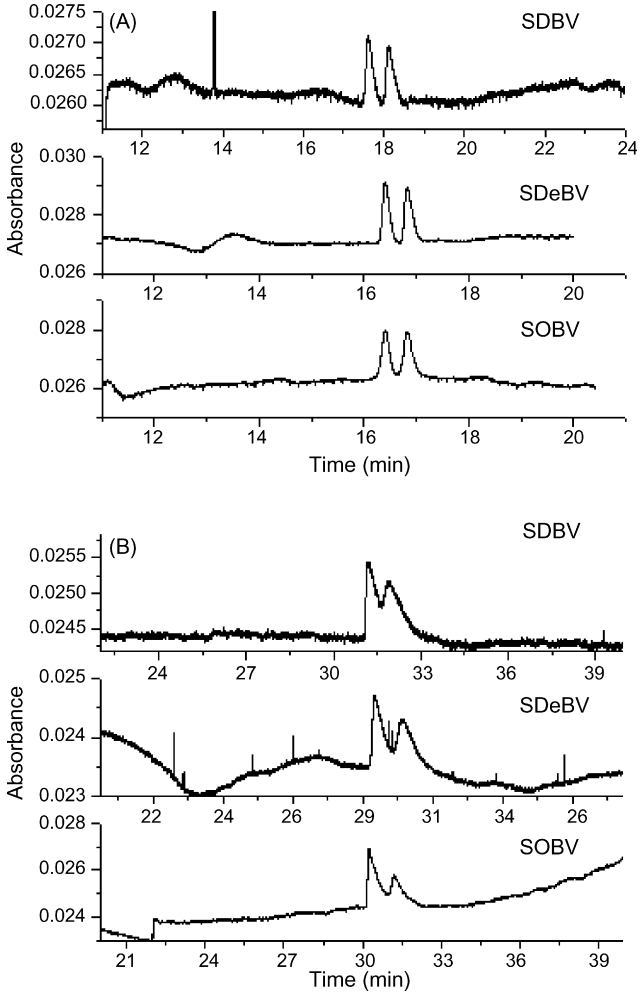


Fig. 4. Electropherograms obtained for enantiomeric separation of BZN (A) and TB (B) using SDBV, SDeBV, and SOBV as chiral selectors. Separation conditions: 60 mM borate buffer, pH 10.3 with 6 mM surfactant for BZN and 50 mM borate buffer, pH 10.3 with 4 mM surfactant for TB. The applied voltage is 15 kV.

Table 4
EOF migration time (t_o), migration time of first enantiomer (t_1), selectivity (α), and resolution (R_s) values for enantiomeric separation of BOH, BNP, BZN, and TB using three chiral selectors SDBV, SDeBV, and SOBV

Analyte	SDBV	SDeBV	SOBV
BOH			
t_o	7.86	7.87	7.92
t_1	43.95	39.22	34.52
α	1.09	1.10	1.09
R_s	5.23	3.70	3.65
BNP			
t_o	9.93	10.05	10.11
t_1	43.77	48.64	52.20
α	1.07	1.06	1.08
R_s	3.04	2.73	3.10
BZN			
t_o	10.14	10.02	10.38
t_1	15.92	16.41	16.41
α	1.03	1.02	1.02
R_s	1.61	1.60	1.50
TB			
t_o	10.14	9.22	9.36
t_1	30.68	29.72	30.24
α	1.02	1.03	1.03
R_s	1.06	1.26	1.67

For separation conditions, see text.

migration time of EOF (t_o), migration time of first enantiomer (t_1), α, and R_s in each case are listed in Table 4. Some of the salient features of the data presented in Table 4 are (i) the t_1 values obtained for BOH using the three surfactants decreases in the order SDBV > SDeBV > SOBV and for BNP the order is reversed, (ii) the α value for any particular analyte (BOH, BNP, BZN, or TB) using the three different chiral selectors are practically same, and (iii) the R_s value obtained for the analytes using the three chiral selectors are comparable except for BOH for which a slight higher resolution can be obtained using SDBV. The above results can be explained taking into account the type of aggregates formed by the surfactants in buffer solution, i.e. vesicles or micelles and the physical properties like micropolarity of the aggregates. Vesicles are larger in size compared to micelles and pose more solubilizing ability for the hydrophobic compounds. So the analytes are expected to interact more strongly with the vesicles compared to micelles. This is reflected in the larger t_1 values for BOH using SDBV compared to the other two surfactants. BZN, and TB being relatively small molecules and less hydrophobic, get almost equally partitioned between the vesicular and miceller phase. The low t_1 value obtained for BNP using SDBV is due to the high charge density of the vesicles. BNP is negatively charged in the working pH range and the electrostatic repulsion at the vesicle surface decreases it's partitioning with the vesicles. It is well known that chiral recognition occurs because of interaction of the analyte with the chiral selector near the stereogenic center. The chiral head group of SDBV, SDeBV and SOBV is same. Therefore, the α values obtained for the analytes using the three surfactants are nearly same. However, the R_s values obtained for each analyte using the three chiral selectors are slightly different owing to the difference in retention times.

4. Conclusion

In summary, two micelle-forming amino acid based surfactants SDeBV and SOBV having different hydrophobic chain length were used as chiral selectors for enantiomeric separation of chiral compounds BOH, BNP, BZN, and TB. The results of this study were compared with our earlier published results of vesicle-forming surfactant SDBV to evaluate the effect of hydrophobic chain length on enantioselectivity. The results obtained from the above studies indicate that the morphology of the self-assemblies formed by the surfactants has no significant effect on enantioselectivity of the surfactants when used as chiral selectors in MEKC. The type of aggregate formed, however, have effect on other chromatographic parameters like analysis time, retention factor, and resolution.

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